Contents lists available at ScienceDirect
Asian Pacific Journal of Tropical Medicine
journal homepage:www.elsevier.com/locate/apjtm

Document heading doi:

Asymptomatic malaria parasitemia does not induce additional oxidative stress in pregnant women of South East Nigeria

Uchenna Ifeanyi Nwagha[1*], Tochukwu Christopher Okeke[1], Theresa Ukamaka Nwagha[2], Fidelis Ebele Ejezie[3], Sylvester Ogbonna Ogbodo[4], Cyril Chukwudi Dim[1], Bond Ugochukwu Anyaehie[5]

[1]*Department of Physiology/Obstetrics and Gynaecology, College of Medicine, University of Nigeria, Enugu Campus, Nigeria*
[2]*Department of Haematology/Immunology, College of Medicine, University of Nigeria, Enugu Campus, Nigeria*
[3]*Department of Medical Biochemistry, College of Medicine, University of Nigeria, Enugu Campus, Nigeria*
[4]*Department of Medical Laboratory Sciences, College of Health Sciences, Ebonyi State University, Abakaliki, Nigeria*
[5]*Department of Physiology, College of Medicine, University of Nigeria, Enugu Campus, Nigeria*

ARTICLE INFO

Article history:
Received 25 October 2010
Received in revised form 27 November 2010
Accepted 15 January 2011
Available online 20 March 2011

Keywords:
Oxidative stress
Asymptomatic malaria parasitemia
Antioxidants vitamins
Pregnancy
Nigeria

ABSTRACT

Objective: To determine the relationship between asymptomatic malaria parasitemia and some oxidative stress parameters in pregnant Nigerian women. **Methods:** This is a cross-sectional study involving 130 normal pregnant women at various trimesters, who were attending antenatal clinic at the University of Nigeria Teaching Hospital (UNTH) and Kenechukwu Specialist Hospital in Enugu. A comparable group (control), made of 30 non pregnant women was also recruited. After a 24 hour dietary recall, serum levels of vitamin A, C and malondialdehyde (MDA) were determined by colorimetric method, while vitamin E was determined by absorptiometric method. **Results:** There were no statistically significant differences in age, parity, estimated calorie, vitamins A, C and E intake between the pregnant and non pregnant groups (P> 0.05). The serum level of the vitamins (umol/L) and MDA (umol/L) in control, 1st, 2nd and 3rd trimesters respectively were: (1)Vitamin A: 1.6±0.36 vs 0.6±0.26 vs 0.62± 0.33 vs 0.46± 0.21 (P < 0.0001); (2) Vitamin C: 75.65±14.15 vs 62.97±24.4 vs 37.85±15.19 vs 28.94±8.52 (P<0.0001); (3) Vitamin E: 3.01 ± 1.32 vs 3.45±2.01 vs 9.36±2.75 vs 9.82±2.97 (P<0.0001); (4) MDA: 1.42± 0.02 vs 1.61±0.02 vs 1.79 ±0.02 vs 2.03±0.05 (P<0.0001). However, there were no significant changes in the serum level of the vitamins and MDA between the positive and the negative parasitemia subjects (P>0.05). **Conclusions:** Asymptomatic malaria parasitemia does not induce additional oxidative stress on pregnant women in Nigeria. The enormity of acute and complicated attack should be further investigated.

1. Introduction

Pregnancy is a physiological process that is characterized by dynamic changes in multiple organ systems. Ultimately, it leads to an increase in basal oxygen consumption and changes in energy substrate use by different organs, including the feto-placental unit[1, 2]. Some of these changes that occur during pregnancy induce oxidative stress, and this has been recognized as an important feature of many diseases[2, 3]. Oxidative stress occurs when reactive oxygen species (ROS) generation exceeds available antioxidant defenses[2]. During pregnancy, the high metabolic energy demand increases oxygen requirement and accordingly, predisposes the body to increased risk of oxidative stress[4]. ROS leads to the damage of nucleic acid, proteins and lipids. Damage to poly unsaturated fatty acids commonly lead to the production of malondialdehyde (MDA); which has widely been accepted as a potent oxidative stress indicator[5].

Antioxidants are defense biomolecules and mechanisms that combat the effect of free radical damage. The primary antioxidant enzymes include superoxide dismutase (SOD), glutathione peroxidase and catalase. Vitamins A, C and E, uric acid, albumin, and bilirubin are classified as

*Corresponding author: Dr Uchenna Ifeanyi Nwagha, Department of Physiology, College of Medicine, University of Nigeria, Enugu Campus/Department of Obstetrics and Gynaecology, College of Medicine, University of Nigeria Teaching Hospital Ituku-Ozala, Enugu, Nigera.
E-mail: uchenwagha@yahoo.com; uchenna.nwagha@unn.edu.ng
Tel: +2348033128233

nonenzymatic antioxidants[6]. Vitamin C is an important hydrophilic antioxidant in cells efficiently scavenging a range of reactive oxygen species and may contribute in protecting the fetus from oxygen free radical damage[7-9]. Deficiency of vitamin C has been shown to affect placental structure and facilitate placental infection with increased risk of premature rupture of placental membranes and premature births[10]. It is thus likely that deficiency may induce oxidative stress[11]. Vitamin E is a lipophilic free radical scavenger antioxidant, which interferes with lipid peroxidation[12]. It is a known fact that vitamin C and E act synergistically against oxidative stress because vitamin C is involved in the regeneration of oxidised vitamin E[13]. Vitamin A (retinol compounds) are fat soluble anti oxidant micronutrients, essential for many biological functions including vision, reproduction, growth and development[14]. Indeed vitamin A deficiency has been implicated in the etiopathogenesis of fetal malformations, intrauterine growth retardation (IUGR), and spontaneous abortion[16-18]. Oxidative stress in pregnancy tends to reduce the level of some of these vitamins as they are quickly mobilized to thwart peri oxidative damage. Consequences of a failed mechanism include; reduced placental efficiency and calcification, fetal malformation preeclampsia and eclampsia, recurrent abortion, intrauterine growth restriction and gestational diabetes[19-22].

Malaria in pregnancy is a major cause of fetal and maternal morbidity and mortality. Studies have shown that symptomatic malaria induces additional oxidative stress in pregnancy[23, 24]. Similarly, one would expect asymptomatic malaria parasitemia to do the same since it is froth with several complications[25]. The consequence of this assumption is the reduction in the plasma level of available antioxidant vitamins as they are mobilized as defense molecules to counteract oxidative damage.

In Nigeria little attention has been paid to the relationship between antioxidants, oxidative stress and malaria in pregnancy. As a result, the effect of asymptomatic malaria parasitemia on the level of some oxidative stress indictors in our pregnant women was evaluated.

2. Material and methods

2.1. Study design and setting

This is a cross sectional study involving 130 normal pregnant women at various trimesters (34 in first trimester, 44 in second trimester and 52 in third trimester) attending antenatal clinic at University of Nigeria Teaching Hospital (UNTH) and Kenechukwu Specialist Hospital in Enugu. The control group was made up of 30 non pregnant women recruited from the staff of the above named institutions. Subjects with febrile conditions, multiple pregnancy, preeclampsia, diabetes mellitus, and chronic renal disease, sickle cell anemia and HIV infections were excluded from the study. After obtaining ethical clearance and informed verbal consent [Ethical clearance; University of Nigeria Teaching hospital, Enugu ethical committee, (UNTH/CSA.329/VOL6)], the subjects that met the above criteria were randomly recruited. We obtained personal history, history of present pregnancy, past obstetric history, past medical history, family and social history and review of systems were obtained. The gestational age was assessed from the last normal menstrual period (LMP). Trimester was defined as first trimester (< 14 weeks); second (14- 27 weeks) and third (> 27 weeks). Those that were not sure of their LMP were dropped. All the women were on routine iron and folic acid supplementation but not on vitamin A, E or C. A 24 hour dietary history was obtained and caloric as well as vitamins A, E and C contents were estimated using the nutrient composition of commonly eaten foods in Nigeria[26] and other parts of the world[27].

Medical and obstetric examinations were performed. Axillary temperature was taken to exclude fever and temperature of less than 37.5 ℃ was considered as normal.

2.2. Estimation of malaria parasites

After cleaning the volar surface of the arm with cotton wool moistened with methylated spirit, peripheral blood samples were collected in sterile containers. Thin and thick blood smears were made from each of these samples, stained with Geimsa and then examined under the microscope using x100 objective lens in each case. Identification of species was done using the thin blood smear. The parasite density was estimated on the thick smear under oil immersion and viewed using x100 objective lens. The determination was by counting the number of asexual forms of *Plasmodium falciparum* parasites against at least 100 leucocytes and 200 leucocytes for definitive count. The number of asexual parasites was calculated using the following formula.

Parasites/ μ L= No of asexual parasites × 8000 leucocytes/200 leucocytes

.

A negative result was recorded after thorough examination of 100 fields without any parasite. Quality control was ensured by using freshly reconstituted and filtered Geimsa stains. The microscopist is very experienced and spent an average of 15 minutes to one hour on each thick and thin film respectively. Comparison was made with both known positive and negative thin films. All the laboratory analyses were done on the day of sample collection except microscopy. All the patients with malaria parasites were promptly and adequately treated free of charge.

2.3. Estimation of antioxidant vitamins

The remaining samples were immediately put into sterile plain bottles. Samples were allowed to stand for about 30 minutes to clot and then centrifuged at 3 500 rpm for ten minutes. The serum was collected and kept frozen in the refrigerator at a temperature of -20 ℃. Vitamin

C was determined by colorimetric method using dinitro phenyl-hydrazine[28], while vitamin E was determined by the absorptiometric method in ferric chloride oxidation reaction[29]. Vitamin A was estimated colorimetrically using Tri fluoro-acetic Acid (TFA)[30]. Serum malondialdehyde (MDA) was measured using the colorimetric method[31].

2.4. Statistical analysis

This was done using SPSS version 11 computer software. Results were presented as mean and standard deviation. One Way Analysis of Variance (ANOVA) and Turkey's post hoc where applicable was used to test for differences in the level of the parameters among all the subjects as pregnancy progressed. The student *t*- test was used to determine the differences in the parameter between malaria positive and malaria negative pregnant women. *P* values $\leqslant$0.05 were considered significant.

3. Results

Table 1 shows some sociodemographic variables of the subjects. Majority of the subjects was within the age range of 21 to 30 years; and was civil servants who attended secondary and tertiary institutions. The mean parity, gestational age (GA), estimated daily calorie and vitamin intake are shown in Table 1. All the women were approximately on the same calorie and vitamin intake (*P*>0.05).The parasite density of the malaria positive women were either mild or moderate. Expectedly, the GA showed a significant change between the trimesters (*P*< 0.05). Table 2 shows the serum level of the vitamins and MDA.

3.1. Vitamin A

The serum level -of vitamin A (μ mol/L) was 1.63±0.36 in nonpregnant subjects. This value decreased to 0.63± 0.26 in the first trimester, 0.62±0.33 in the second trimester and 0.46±0.21 in the third trimester. These changes are statistically significant (*P*<0.0001); however post hoc multiple comparison shows that the difference observed is as a result of the serum level of all the pregnant subjects compared with the non pregnant control; there were no statistically significant changes as pregnancy progressed (*P*=0.07). Furthermore, the differences between the serum level of vitamin A among the malaria negative and malaria positive subjects in all the trimesters and control were not statistically significant (1st trimester: *P*=0.97, 2nd trimester: *P*=0.15, 3rd trimester: *P*=0.86 and control *P*=0.89).

Table 1
Mean age, parity and some estimated nutrient intake.

Variables	1st trimester	2nd trimester	3rd trimester	Control	*P*
Age (yrs)	30.1±5.6	28.2±5.5	29.0±4.0	28.2±6.3	0.67
Parity	1.0±1.4	1.2±1.7	1.9±1.5	1.5±1.8	0.54
GA (weeks)	9.9±1.8	21.3±3.8	34.4±2.9	0.0±0.0	0.0001
EDCI (kcal)	2160.0±110.0	2150.0±108.0	2170.0±112.0	2160.0±110.0	0.58
EDVAI(μ moL)	24.0±4.0	26.0±5.0	25.0±4.0	26.0±5.0	0.72
EDVCI (μ moL)	3970.0±26.0	3800.0±22.0	3760.0±20.0	3820.0±26.0	0.39
EDVEI (μ moL)	14.0±2.0	16.0±3.0	16.0±3.0	15.0±2.0	0.78

EDCI = Estimated daily calorie intake, EDVAI = Estimated daily vitamin A intake. EDVCI = Estimated daily vitamin C intake, EDVEI= Estimated daily vitamin E intake, GA=Gestational age.

Table 2
Serum level of some oxidative stress parameters.

Variables (μ mol/L)	MP	Control	1st trimester	2nd trimester	3rd trimester
Vitamin A	$AllS^{bjs}$	1.63±0.36	0.63±0.26	0.62±0.33	0.46±0.21
	MP^{-ve}	1.63±0.37	0.70±0.20	0.61±0.35	0.46±0.22
	MP^{+ve}	1.62±0.35	0.58±0.29	0.63±0.32	0.45±0.21
Vitamin C	$AllS^{bjs}$	75.65±14.15	62.97±24.40	37.85±15.19	28.94±8.52
	MP^{-ve}	74.90±14.50	59.20±24.40	38.40±14.51	29.20±8.92
	MP^{+ve}	79.90±12.10	65.60±24.70	36.80±21.61	28.30±7.60
Vitamin E	$AllS^{bjs}$	3.01±1.32	3.45±2.01	9.36±2.75	9.82±2.97
	MP^{-ve}	2.80 ±1.11	3.12±1.30	9.20±3.10	9.90±2.80
	MP^{+ve}	3.30 ±1.52	4.01±2.80	9.50±2.50	8.90±4.20
MDA	$AllS^{bjs}$	1.42±0.02	1.61±0.02	1.79±0.02	2.03±0.05
	MP^{-ve}	1.44±0.02	1.60±0.02	1.81±0.02	2.07±0.05
	MP^{+ve}	1.41±0.02	1.62±0.02	1.77±0.02	2.01±0.05

Values are mean ± SD, $AllS^{bjs}$ = All the subjects, MP^{-ve} =Negative malaria parasite, MP^{+ve} =Positive malaria parasite.

3.2. Vitamin C

The serum level of vitamin C (μ mol/L) was 75.65±14.15 in nonpregnant subjects. This value decreased to 62.97±24.4 in the first trimester. 37.85±15.19 in the second trimester and 28.94±8.52 in the third trimester. These changes were statistically significant (P<0.0001). Post hoc analysis however did not observe any difference between the 2nd and the 3rd trimester (P=0.08).There were no significant differences in serum level between the malaria negative and malaria positive subjects (1st trimester: P=0.48, 2nd trimester: P=0.46, 3rd trimester: P=0.80 and control=0.72).

3.3. Vitamin E

The serum level of vitamin E (μ mol/L) was 3.01±1.32 in nonpregnant subjects. This value increased to 3.45±2.01 in the first trimester, 9.36±2.75 in the second trimester and 9.82 ±2.97 in the third trimester. These changes are statistically significant (P<0.0001). However, the post hoc test did not show any difference between the control versus 1st trimester and the 2nd versus 3rd trimester (P=0.88 and P=0.81 respectively). There were no significant differences in serum level of vitamin E between the malaria negative and malaria positive subjects (1st trimester: P=0.67, 2nd trimester: P=0.78, 3rd trimester: P=0.27 and control=0.29).

3.4. Malondialdehyde (MDA)

The serum level of MDA (μ mol/l) was 1.42±0.02 in nonpregnant subjects. This value increased to 1.61±0.02 in the first trimester, 1.79±0.02 in the second trimester and 2.03 ±0.05 in the third trimester. These changes are statistically significant (P<0.0001). However, there were no significant differences in serum level of MDA between the malaria negative and malaria positive subjects (1st trimester: P=0.69, 2nd trimester: P=0.68, 3rd trimester: P=0.57 and control=0.59).

4. Discussion

Understandably, pregnancy is associated with high metabolic demand and elevated requirement for tissue oxygen, which result in increased oxidative stress and mobilization of antioxidant defense[32]. In this study serum levels of vitamins A and C were found to be lower in pregnant women while that of vitamin E and MDA were found to be higher in pregnant than non pregnant women. These findings are in conformity with results from other studies[33-37]. Although studies have found low antioxidant capacity in some Nigerian women, it is important to note that oxidative processes are not entirely harmful in normal pregnancy as they exert fundamental regulatory function during pregnancy[37].

The present study showed statistical significant decrease each in the serum level of ascorbic acid (vitamin C) and vitamin A from 1st trimester to 3rd trimester compared with control. There was also a steady decline in the serum levels of these antioxidants with increasing gestational age. MDA, which is a common end product of oxidative damage significantly increased as pregnancy progressed. The appreciable lower levels of these antioxidants during pregnancy are basically due to their utilization as defense mechanism against reactive oxygen species/free radicals during oxidative stress and fetal growth[7, 8]. Other factors that may be responsible for the reduction in some serum antioxidant levels include hemodilution of pregnancy and active transfer of ascorbic acid from mother to the fetus[2]. Vitamin E, although an antioxidant did not show any sign of depletion, instead there was a steady rise as pregnancy progressed. This pattern, as demonstrated in another study[35], is explicable, as the hyperlipidemic state of pregnancy ensures the enhancement of vitamin E; a naturally occurring fat soluble vitamin[38]. Furthermore, it seems that there is a positive balance between vitamin E metabolism in pregnancy and its utilization as defense mechanisms for oxidative stress. Indeed, it is probable that vitamin E enhancement overshadows its utilization as anti oxidant defense molecule.

There is no doubt that oxidative stress exist in pregnancy, as well as symptomatic malaria[24, 25]. However, in this study, the relationship between some antioxidant vitamin levels, MDA and asymptomatic malaria parasitemia was such that one would readily conclude that additional oxidative stress did not occur, irrespective of the numerous fetomaternal complications associated with asymptomatic parasitemia[23]. In the interim, since there is paucity of literature for or against our findings, we advocate that until such arise, asymptomatic malaria parasitemia does not stimulate additional oxidative stress in pregnant Nigerian women. It is a possibility that if we had categorized the degree of asymptomatic parasitemia as mild or moderate, matched against some oxidative stress indicators, an interesting scenario may pop up. Probably, if we had analyzed other oxidative stress indicators, the story may be different. However, this is a stepping stone towards further research on asymptomatic malaria parasitemia and oxidative stress in pregnant Nigerians.

Conflict of interest statement

We declare that we have no conflict of interest.

References

[1] Sies H. What is oxidative stress? In: Keaney JF (ed). Oxidative stress and vascular disease. Boston: Kluwer Academic Publishers;2000,p.1-8.

[2] Ejezie FE, Onwusi EA, Nwagha UI. Some biochemical markers of oxidative stress in pregnant Nigerian women. *Trop J Obstet*

Gynaecol 2004; **21**(2): 122– 124.
[3] Halliwell B. Antioxidants in human health and disease. *Annu Rev Nutri* 1996;16: 33– 50.
[4] Scott KP, Shannon LL. Analysis of cellular responses to free radicals: Focus on exercise and skeletal muscle. *Proc Nutri Soc* 1999; **58**: 1025–1033.
[5] Nielsen F, Mikkelsen BB, Nielsen JB, Andersen HR, Grandjean P. Plasma malondialdehyde as biomarker for oxidative stress; reference interval and effects of life–style factors. *Clin Chem* 1997; **43**:1209–1214.
[6] Guerin P, El–Mouatssim S, Menezo Y. Oxidative stress and protection against reactive oxygen species in the pre–implantation embryo and its surrounding. *Hum Reprod Updt* 2001; **7**: 175–189.
[7] Garry GD. Determination of activity of antioxidants in human subjects. *Proc Nutri Soc* 2001; **58**: 1015–1024.
[8] Rose RC. Ascorbic acid metabolism in protection against free radicals a radiation model. *Biochem Biophys Res Commun* 1990; **169**: 430–436.
[9] Cederberg J, Siman CM, Eriksson UJ. Combined treatment with vitamins E and C decreases oxidative stress and improves fetal outcome in experimental pregnancy. *Pediatr Res* 2001; **49**: 755–762.
[10]Casanueva E, Viteri FE. Iron and oxidative stress in pregnancy. *J Nutr* 2003; **133**: 1700S– 1708S.
[11]Mario M, Jerry MR. Plasma ascorbic acid levels in pregnancy. *Am J Obstet Gynaecol* 1971; **109**: 960–965.
[12]DiMascio P, Murphy ME, Sies H. Antioxidant defense systems: the role of carotenoids tocopherols and thiols. *Am J Clin Nutr* 1991; **53** (1): S194– S200.
[13]Parker L. Interactions among antioxidants in health and disease; vitamin E and its redox cycle. *Proc Soc Exp Biol Med* 1992; **200**:271–276.
[14]Blomhoff RB, Green MT, Green JB, Berg T, Norum KR. Vitamin A metabolism: new perspectives on absorption, transport and storage. *Physiol Rev* 1991; 71: 951–990.
[15]Underwood BA. Vitamin A in human nutrition; public health. In: Sporn MB (ed). *The retinoid: biology, chemistry and medicine*. 2nd ed. New York: Raven Press; 1994,p.211–229.
[16]Shah RS, Rajalakshmi R. Vitamin A status of the newborn in relation to gestational age, body weight, and maternal nutritional status. *Am J Clin Nutr* 1984; **40**: 794–800.
[17]Shah RS, Rajalakshmi R, Bhatt RV, Hazra MN, Patel BC, Swamy NB, et al. Liver stores of vitamin A in human fetuses in relation to gestational age, fetal size and maternal nutritional status. *Br J Nutr* 1987; **58**:181–189.
[18]Neela J, Raman L. The relationship between maternal nutritional status and spontaneous abortion. *Natl Med J India* 1997; **10**: 15–16.
[19]Woods JR (Jnr). Reactive oxygen species and preterm premature rupture of membranes a review. *Placenta* 2000; **22**: 538– 544.
[20]Poston L, Chapell LC. Is oxidative stress involved in the aetiology of preedampsia? *Acta Paed Suppl* 2001; **90**: 3–5.
[21]Vural P, Akgul C, Yildirin A, Canbaz M. Antioxidant defense in recurrent abortion. *Clin Chim Acta* 2000; **295**:169–77.
[22]Karowiez–Bilinska A, Suzin J, Sieroszewski C. Evaluation of oxidative stress indices during treatment in pregnant women with intrauterine growth retardation. *Med Sci Monit* 2002; **8**: 211–216.
[23]Ifoue SHT, Mofor TC, Gouado I, Teto G, Asonganyi T, Amvam PH. Evaluation of oxidative stress and antioxidant status of pregnant women suffering from malaria in Cameroon. *Ind J Clinl Biochem* 2009; **24**(3): 288–293.
[24]Becker K, Tilley L, Vennerstrom JL, Roberts D, Rogerson S, Ginsburg H. Oxidative stress in malaria parasite–infected erythrocytes: host–parasite interactions. I*nt J Parasitol* 2004; **34**: 163 –189.
[25]Nwagha UI, Ugwu VO, Nwagha TU, Anyaehie USB. Asymptomatic Plasmodium parasitaemia in pregnant Nigerian women: Almost a decade after the roll back malaria. *Trans R Soc Trop Med Hyg* 2009; **103**:16–20.
[26]Oguntano EB, Akinyele IO. Nutrient composition of commonly eaten foods in Nigeria–raw processed and prepared. Ibadan: Food Basket Foundation Publication;1995.
[27]Paul AA, Southgate DAT (eds). *The composition of foods*. 4th ed. London: Her Majesty Stationery Office (HMSO);1979.
[28]Varley H Vitamins. In: Varley H, Gowenlock AH, Bell M (eds). Practical clinical biochemistry. hormones, vitamins, drugs and poisons. London:Williams Heinemann Medical Books ltd;1976,p.607–638.
[29]Pearson D. Oil and fats. In: Egan H (ed). *The chemical analysis of foods*. 8th ed. Edinburgh: Churchill Livingstone; 1980,p.517–537.
[30]International Vitamin A Consultation Group (IVACG). *Biochemical methodology for the assessment of vitamin A status*. Washington: IVACG; 1982,p.24–30.
[31]Satoh K. Serum lipid peroxide in cerebrospinal disorder determined by a new colorimetric method. *Clin Chim Acta* 1978; **40**: 37–43,
[32]Knapen MFCM, Zusterzeel PLM, Peters WHM, Steegers EAP. Glutathione and glutathione related enzymes in reproduction. *Eur J Obstet Gynecol Reprod Biol* 1999; **82**: 171–184.
[33]Christian P, West KP Jr, Khatry SK, Katz J, Shrestha SR, Pradhan EK, et al. Night blindness of pregnancy in rural Nepal-nutritional and health risks. *Int J Epidemiol* 1998; **27**: 213–237.
[34]Nwagha IU, Ejezie FE. Serum ascorbic acid levels during pregnancy in Enugu, Nigeria. *J Coll Med* 2005; **10**(1): 43–45.
[35]Von Mandach U, Huch R, Huch A. Maternal and cord serum vitamin E levels in normal and abnormal pregnancy. *Int J Vitam Nutr Res* 1994; **64**(1): 26–32.
[36]Olayaki LA, Ajao SM, Jimoh GA, Aremu IT, Soladoye AO. Effect of vitamin c on malondialdehyde (MDA) in pregnant Nigerian women. *J Bas Appl Sci* 2008; **4**(2): 105–108.
[37]Idogun ES, Odiegwu ME. Momoh SM, Okonofua FE. Effect of pregnancy on total antioxidant capacity in Nigerian women. *Pak J Med Sci* 2008; **24**(2): 292–295.
[38]Herrera E, Ortega H, Alvino G, Geovannini N, Cetin I. Relationship between plasma fatty acid profile and antioxidant vitamins during normal pregnancy. *Eur J Clin Nutri* 2004; **58**: 1231–1238.
[39]Kharb S, Gulati N, Ghalaut VS, Singh GP. Vitamin E concentration in normal pregnant women. *J Obstet Gynecol Ind* 2000; **50**: 48–49.
[40]Kankofer M. Nonenzymatic antioxidative defense mechanism against reactive oxygen species in bovine retained and not retained placenta; vitamin C and glutathione. *Reprod Domest Anim* 2001; **36**: 203–6.